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Immune Response of Cattle Vaccinated Simultaneously with FMD and Rabies Vaccines

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The present study was undertaken to evaluate the immune response of cattle following simultaneous vaccination with foot and mouth disease and rabies vaccines. A binary ethylene amine BEI inactivated FMD vaccine and an inactivated rabies vaccine were used and antibody response of cattle was evaluated using a LPB ELISA and indirect ELISA. For immunization cattle were grouped into I, II, III and IV and simultaneous vaccination was carried out in Gp I. Gp II was vaccinated against FMD, Gp III against rabies separately. Gp IV was kept as unvaccinated control. On the day of vaccination, all the groups were sero-negative against FMD virus and rabies virus. The percentage of animals showing protective antibody titre against FMDV types O, A and Asia-1 at 15, 30, 60 and 90 days post vaccination were 40-30-50, 90-80-90, 90-80-80 and 80-70-70 in group I, and 60-40-50, 100-90-90, 90-80-80 and 70-70-80 in group II, respectively. The percentage of animals showing protective antibody level against rabies virus at 15, 30, 60 and 90 days post vaccination were 90, 100, 100 and 90 in group I, and 100, 100, 100 and 90 in group III, respectively. Thus the present investigation revealed that foot and mouth disease vaccine had no adverse influence on the production of antibodies against rabies vaccine. The simultaneous immunization of cattle with foot and mouth disease vaccine and rabies vaccines elicited similar antibody response against the different vaccine virus types and rabies virus, compared to antibody response of separate vaccination against FMD and rabies.

Key words: FMD virus, Rabies virus, LPB ELISA, INDIRECT ELISA.

Foot and mouth disease (FMD) is probably the most important livestock disease in the world in terms of economic impact, which is primarily limited to cloven-footed domesticated animals, especially cattle, sheep, goat, pigs and buffalo (Kumar *et al.* 1994). Foot and mouth disease is caused by the Foot and Mouth Disease virus of the genus Aphthovirus under the family Picornaviridae. There are seven immunologically distinct serotypes of the virus, namely O, A, C, Asia-1, SAT1, SAT2 and SAT3 that infect cloven-footed animals (Sewell and Brocklesby 1990). The serotypes O, A, and Asia-1 are endemically prevalent in the country including the North-Eastern states (Dutta *et al.* 1984). Among the livestock at risk, cattle are the primary susceptible host to foot and mouth disease (Mann and Sellers 1990). Thus, due to the highly contagious nature and economic importance of FMD, it can be said that in countries where eradication is not feasible, vaccination of livestock is clearly economically beneficial.

Rabies in bovines also remains as a serious economic problem in a tropical country like India as cattle here are used for milk production and in agricultural operations. Rabies is a viral

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zoonosis and carnivores such as stray dogs and foxes are hosts of rabies virus in nature. The disease is caused by the Rabies virus, which is a negative-stranded RNA virus of the family Rhabdoviridae (King and Turner1993). More than 2.5 thousand million people live in regions where rabies is endemic. It is estimated that each year at least 50,000 people die from rabies, and more than 10 million receive post-exposure vaccination against the disease. Thus, prevention of exposure to the rabies virus and of infection after exposure is of paramount importance in preventing mortality due to rabies. Regular prophylactic vaccination of dairy animals against infectious diseases in developing countries has become an important input to maintain milk production and to reduce economic losses.

Keeping in view the highly contagious nature and economic importance of FMD and the zoonotic importance of rabies, it can be said that vaccination against both the diseases should be given top most priority. In an exercise to reduce the labour and the cost of vaccination, many workers attempted combined vaccination against FMD and rabies in different parts of the world. Thus, the present study was undertaken to evaluate the antibody response of cattle vaccinated simultaneously with FMD and Rabies vaccines.

MATERIALS AND METHODS

Experimental animals

A total of 40 apparently healthy cattle of different age groups belonging to private organised cattle herds were selected. There was no history of foot and mouth disease outbreaks or rabies cases in the selected cattle herds and no vaccination against FMD or rabies was carried out for a period of one year prior to the present study. All the animals were crossbred animals having germplasm of either Jersey or Holstein-Friesian or Sindhi. Experimental animals were divided into four groups – groups I, II, III and IV, comprising of 10 animals in each group

Vaccines

The binary ethylene amine (BEI) inactivated oil adjuvanted tetravalent (O, A, C and Asia-1) FMD vaccine (Clovax) prepared by Intervet India Pvt. Ltd., Pune was used for vaccination against FMD in the present experiment. Vaccination against rabies was carried out with inactivated (cell culture) Rabies Veterinary Vaccine adjuvanted with aluminium hydroxide gel and marketed as Megavac-R manufactured by Indian Immunologicals Ltd. A dose of 3 ml of the FMD vaccine was administered subcutaneously to each animal irrespective of age as per the manufacturer's guidelines. Whereas, a dose of 1 ml of the rabies vaccine was administered intramuscularly to each animal, irrespective of age as per the manufacturer's guidelines for rabies vaccination.

Vaccination schedule

The animals of group I were inoculated with FMD tetravalent vaccine and rabies inactivated cell culture vaccine simultaneously at two different sites. The animals of group II were vaccinated with FMD vaccine alone, while the animals of
group III were vaccinated with rabies vaccine alone. The animals of group IV were kept as unvaccinated control.

Collection of serum samples

Blood samples were collected by jugular vein-puncture from each of the vaccinated and control animals and serum was separated and stored at - 20°C without addition of any preservative for further use. The serum samples were collected on '0' day (prior to vaccination) and thereafter at 15^{th}, 30^{th}, 60^{th} and 90^{th} days post vaccination.

Foot and mouth disease virus

Reference FMD virus types O, A and Asia-1 were obtained from the Central FMD virus Typing Laboratory, Indian Veterinary Research Institute, Mukteswar, India. The virus titre was measured by sandwich ELISA. Tissue culture fluid showing 1 OD in the ELISA reader (Bio-Rad) was used for liquid phase blocking ELISA.

Propagation of the FMD vaccine virus

For the propagation of FMD vaccine viruses BHK-21 cell line (National Centre for Cell Science, Pune) was used. The cells were grown in Eagle minimum essential medium (Hyclone, USA) supplemented with 10% fetal bovine serum, 2 mM L-glutamine , Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM non-essential acids, 1.0 mM sodium pyruvate, 100IU of penicillin G per ml and 100 ug of streptomycin per ml. Cells were routinely subcultured with 0.25% trypsin

(1:250). Subculture of BKH-21 cell monolayer was done as per the method described by Suryanarayana *et al.* (1982). The maintenance medium used for infection of cell monolayers consisted of the same medium (MEM, Eagle) supplemented with 2 per cent foetal calf serum.

Titration of the virus

Titration of each of the types of the virus was done by determining the virus concentration in the cell culture fluid by sandwich ELISA as described in the protocol supplied by Central FMD Typing Laboratory, Mukteswar, India. Cell culture fluid showing 1 OD in the ELISA reader (Bio-Rad) was used for the test proper.

Preparation of hyperimmune serum against rabies virus

Megavac-R (Indian Immunologicals, Hyderabad) was used to prepare the hyperimmune serum in rabbit. One ml of vaccine was given intramuscularly as the first injection. Subsequently, three doses of Megavac-R vaccine were given at weekly intervals. Seven days after the last injection, rabbit blood was collected for serum separation. Serum was stored at - 20°C. This serum was used as the positive control serum in the indirect ELISA.

Assay of antibody

Antibody titre of the serum samples was screened by liquid phase blocking ELISA against FMD virus serotypes and by indirect ELISA against rabies virus.

Liquid phase blocking ELISA (LPBE)

The liquid phase blocking ELISA was performed according to the method described by Central FMD virus Typing Laboratory, Mukteswar, India, which was a modification of the method of Hamblin *et al.* (1987). Serum samples collected from vaccinated cattle were assayed for type-specific antibodies against the FMD virus types O, A and Asia-1 by this test.

Indirect ELISA

Indirect ELISA was performed as per the method described by Piza *et al.* (1999), with slight modification, to determine the level of rabies antibody in the serum samples of vaccinated animals. Sera with OD values greater than 0.282 were considered as corresponding to virus neutralization antibody > 0.5 IU/ml, which was recommended by the WHO working group (World Health Organization, 1992) as the minimum level required for sero-conversion of vaccinated animals against rabies. Highest dilution of serum showing OD value > 0.282 was, therefore, considered as the titre.

Statistical Analysis

The data obtained from the present study were subjected to statistical analysis as per the method described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Antibody response against FMD vaccine

The results of antibody response against FMD virus types O, A and Asia-1 at different days post vaccination are shown in Table 1 to 3. For serological evaluation, serum antibody titre of $^3\log_{10}2.1$, which is equivalent to a serum dilution of 1: 128, was taken as the protective antibody titre against FMD virus specific antibodies as per the findings of Hamblin *et al.* (1987). All the experimental groups of animals including control (groups I, II and IV) were sero-negative on the day of vaccination (Tables 1 to 3) against FMD virus type-specific antibodies. The animals used were neither vaccinated previously nor affected with the disease in the previous year, which might be the reason for the absence of virus-specific antibodies in the experimental animals. At 15 days post vaccination, the antibody response as detected by LPBE revealed that 40 per cent of the vaccinated animals in group I and 60 per cent of the animals in group II possessed protective antibody titre against FMD virus type 'O'. Similarly, 30 per cent and 50 per cent animals in group I and 40 per cent and 50 per cent animals in group II possessed protective antibody titre against type 'A' and 'Asia-1' respectively at 15 days post vaccination. This indicated that at 15 dpv, desirable protection could not be achieved with the oil-adjuvanted FMD vaccine in most of the animals of both the vaccinated groups I and II. This result corroborated more or less with the findings of Chitrovel *et al.* (1997), who also recorded the onset of antibody response to FMD vaccination by 7 dpv, which subsequently increased up to 21 dpv. At 30 days post vaccination, maximum number of animals possessed protective antibody titre in both the groups I and II against all the three FMD virus tested for, *i.e.* 'O', 'A' and 'Asia-1'. In group I, 90, 80 and 90 per cent of animals and in group II, 100,

90 and 90 per cent of animals possessed protective antibody titre against FMD virus types 'O', 'A' and 'Asia-1', respectively at 30 dpv. In the present study, the protective antibody titre was recorded in maximum number of animals at 30 dpv in both the experimental groups I and II against all the virus types. Rahman *et al.* (1987) observed the appearance of the peak titre at 4th to 5th week post vaccination. Chitravel *et al.* (1997) reported protective antibody levels in 84, 88, 92 and 92 per cent of cattle against FMD virus types O, A, C and Asia-1, respectively after 21 days post primary vaccination. It was also observed that maximum numbers of FMD vaccinated animals were protected at 30 days following revaccination. At 30 dpv, the antibody response in groups I, where simultaneous vaccination against FMD and Rabies was done and that of group II, where separate

Table 1. Number of Animals Possessing Protective Antibody Titre (Log Value ³ 2.1) Against Type 'O' FMD Virus at Different Days Post Vaccination In Different Groups

Group	Number of animals with protective antibody titre at different days post vaccination (n = 10)				
	0	15	30	60	90
I (FMD + Rabies)	0^{a}_{A}	4^{a}_{B}	9^{c}_{B}	9^{c}_{B}	8-^{bc}_{B}
II (FMD)	0^{a}_{A}	6^{b}_{B}	10^{b}_{B}	9^{b}_{B}	7^{b}_{B}
IV(Control)	0^{a}_{A}	0^{a}_{A}	0^{a}_{A}	0^{a}_{A}	0^{a}_{A}

Figures in a row and column bearing a common superscript and subscript respectively do not differ significantly.

Table 2. Number of Animals Possessing Protective Antibody Titre (Log Value ³ 2.1) Against Type 'A' FMD Virus at Different Days Post Vaccination In Different Groups

Group	Number of animals with protective antibody titre at different days post vaccination (n = 10)				
	0	15	30	60	90
I (FMD + Rabies)	0^{a}_{A}	3^{a}_{A}	8^{b}_{B}	8^{b}_{B}	7^{b}_{B}
II (FMD)	0^{a}_{A}	4^{b}_{B}	9^{c}_{B}	8^{c}_{B}	7^{bc}_{B}
IV(Control)	0^{a}_{A}	0^{a}_{A}	0^{a}_{A}	0^{a}_{A}	0^{a}_{A}

Figures in a row and column bearing a common superscript and subscript respectively do not differ significantly.

Table 3. Number of Animals Possessing Protective Antibody Titre (Log Value ³ 2.1) Against Type 'ASIA-1' FMD Virus at Different Days Post Vaccination In Different Groups

Group	Number of animals with protective antibody titre at different days post vaccination (n = 10)				
	0	15	30	60	90
I (FMD + Rabies)	0^{a}_{A}	5^{b}_{B}	9^{c}_{B}	8^{bc}_{B}	7^{bc}_{B}
II (FMD)	0^{a}_{A}	5^{b}_{B}	9^{c}_{B}	8^{bc}_{B}	8^{bc}_{B}
IV(Control)	0^{a}_{A}	0^{a}_{A}	0^{a}_{A}	0^{a}_{A}	0^{a}_{A}

Figures in a row and column bearing a common superscript and subscript respectively do not differ significantly.

vaccination against FMD was done, did not differ significantly irrespective of the virus types. However, there was a significant difference between the antibody responses to all the virus types at different days post vaccination.

Analysis of variance showed that the antibody response to simultaneous vaccination (FMD vaccine + Rabies vaccine) in group I did not differ significantly from the antibody response in group II, (Tables 1 to 4). But when the antibody response of groups I and II was compared with that of the unvaccinated control *i.e.* group IV, a highly significant difference was found. Moreover, there was a significant difference between the antibody responses in both the groups I and II to all the three virus types at different days post vaccination (Tables 1 to 4). The protective antibody titre was also recorded at 60 days post vaccination against all the three FMD virus types tested for. In all, 90, 80 and 80 per cent of animals in both the groups I and II showed protective antibody titre against FMDV types 'O', 'A' and 'Asia-1, respectively. Analysis of variance showed that the antibody response at 30 dpv did not differ significantly with the antibody response at 60 dpv against all the three FMD virus types tested for in both the groups. At 90 days post vaccination, the antibody response slightly declined and the percentage of animals showing protective titre also declined from that of the values seen on 60 dpv. Only 80, 70 and 70 per cent of animals in group I showed protective antibody titre against FMDV types 'O', 'A' and 'Asia-1', respectively. While 70, 70 and 80 per cent of animals in group II showed protective titre against FMD virus types'O', 'A' and 'Asia-1' (Tables 1-3). Analysis of variance showed that the antibody response at 90 dpv in both the groups I and II did not differ significantly as compared to the antibody response at 60 dpv against all the three virus types. However, when the antibody response at 90 dpv in both the groups I and II was compared with that of the unvaccinated control group, group IV, a significant difference was seen, although antibody response in the two vaccinated groups, groups I and II did not differ significantly. In the control group, no appreciable level of antibody against FMD virus was detected during the whole experimental period. For evaluation of protective immune response against FMD virus, LPBE was found to be a reliable

Table 4. Analysis of Variance of Protective Antibody Titre Against FMD Virus Types 'O', 'A' And 'Asia-1' In Different Groups Of Cattle At Different Days Post Vacination

Source of variation	d.f.	Type 'O'		Type 'A'		Type 'Asia-1'	
		M.S.	F	M.S.	F	M.S.	F
Group	2	64.27	12.01**	48.80	11.13**	58.07	13.20**
Days	4	20.10	3.76*	17.23	3.93**	17.40	3.95**
Error	8	5.35		4.38		4.40	
Total	14						

*, P < 0.05, **, P < 0.01

Table 5. Number of animals with protective level of antibody against rabies virus in different groups of animals at diffeernt days post vaccination

Groups	No. of animals vaccinated	No. of animals with protective level of antibody at post vaccination days				
		0	15	30	60	90
I (F & R)	10	0^{a}_{A}	9^{b}_{B}	10^{b}_{B}	10^{b}_{B}	9^{b}_{B}
III (R)	10	0^{a}_{A}	10^{b}_{B}	10^{b}_{B}	10^{b}_{B}	9^{b}_{B}
IV (Control)	10	0^{a}_{A}	0^{a}_{A}	0^{a}_{A}	0^{a}_{A}	0^{a}_{A}

Means in a row and column bearing a common superscript and subscript respectively do not differ significantly

technique as per the reports of Hamblin *et al.* (1987) and the negative logarithm value of 2.1 was associated with percentages of protection > 90 per cent against challenge with virus A 87, O1 cas and C 85 (95, 93 and 100%) respectively and 87 per cent of animals were protected against serotype A 79.

Antibody response against rabies vaccine

In the indirect ELISA performed for detection of rabies virus antibody, sera with OD values greater than 0.282 were considered as corresponding to virus neutralisation antibody > 0.5 IU/ml, which was recommended by the WHO Working Group (World Health Organisation, 1992) as the minimum level required for sero-conversion of vaccinated animals against rabies. Highest dilution of the serum showing OD value > 0.282 was, therefore, considered as the titre. The number of animals with protective level of antibody against rabies virus, irrespective of the titre in the three groups of experimental animals at different days post vaccination are shown in Table 5. In the present study, animals of group I were vaccinated simultaneously with FMD and rabies vaccine, animals of group III were vaccinated with rabies vaccine alone and group IV was kept as the unvaccinated control group. On the day of vaccination, all the animals were sero-negative in all the experimental groups of animals, I, III and IV. In the present study, all the animals were tested on the day of vaccination and were found to be free from any detectable level of antibody in serum, which might be due to the fact that the animals were neither vaccinated nor affected with rabies previously. In group I, it was seen that at 15 days post vaccination, out of 10 animals vaccinated, 9 showed protective level of antibody against rabies virus. At 30 days post vaccination, the number of animals showing protective antibody level increased to 100 per cent, *i.e.* all the vaccinated animals showed protective antibody level, and equal number of animals showing protective antibody level was maintained up to 60 dpv. The number of animals showing protective antibody level then declined to 9 again, *i.e.* 90 per cent of the animals showed protective antibody level at 90 days post vaccination. In group III, *i.e.* the group vaccinated alone with rabies vaccine at 15 days post vaccination, all the 10 animals (100%) showed protective antibody level and the number of animals showing protective antibody level was maintained up to 60 days post vaccination. The number of animals showing protective antibody level then declined to 9, *i.e.* 90 per cent of the vaccinated animals showed protective antibody level at 90 days post vaccination. In group IV, *i.e.* the group kept as the unvaccinated control group, all the animals were sero-negative on the day of vaccination and thereafter at 15, 30, 60 and 90 days post vaccination. Ramanna and Srinivasan (1992) also found peak antibody titre on 21 days post vaccination. The titre then gradually reduced on 45th day and was maintained up to 60 days post vaccination. In the present study, it was observed that simultaneously administered FMD vaccine had no any influence on the antibody titre against rabies vaccine. This result was in agreement with the findings of Palanisamy *et al.* (1992) who performed a study where three groups of crossbred calves were immunised with FMD vaccine alone, FMD + rabies vaccines and rabies

Table 7. Number of animals in groups I and III showing protective level of antibody against rabies virus in various dilutions of serum at different days post vaccination

Groups	No. of animals vaccinated	No. of animals with protective level of antibody at post vaccination days				
		0	15	30	60	90
I (F & R)	1: 2	0	9	10	10	9
	1: 4	0	6	10	10	9
	1: 8	0	1	8	8	0
	1: 16	0	0	2	0	0
III (R)	1: 2	0	10	10	10	9
	1: 4	0	10	10	10	9
	1: 8	0	10	10	10	0
	1: 16	0	5	10	6	0
	1: 32	0	1	3	1	0

Table 8. Analysis of variance of mean reciprocal elisa antibody titre

Sources of variation	d.f.	M.S.	F
Group	2	101.22	139.99**
Day	4	28.18	38.97**
Error	143	0.72	

**, P < 0.01

vaccines alone. It was reported that no significantly different serological response was observed in animals administered FMD vaccine alone, rabies vaccine alone or combined FMD and rabies vaccines. At 90 day post vaccination, the antibody titre was seen to be reducing and the number of animals with protective antibody titre also reduced to 90 per cent in both the groups of vaccinated animals (Groups I and III). Bhattacharya and Narayan (1994) also suggested that a booster dose might be given 60-75 days after the primary vaccination with rabies vaccine and then annually or at longer intervals depending upon the vaccines. Number of animals showing protective level of antibody against rabies virus in various serum dilutions at different days post vaccination are shown in Table 6. Analysis of variance (Table 7) showed that there was a significant (P < 0.01) difference in the mean antibody titre among the three groups of animals. Significance difference in antibody titre was also observed between the two vaccinated groups (groups I and III) on 15, 30 and 60 days post vaccination. However, on 90th dpv, there was no significant difference in antibody titre between groups I and III. However, groups I and III did not differ significantly in terms of number of animals with protective level of antibody on 90th day post vaccination. However, in terms of number of animals with protective level of antibody, there was no significant difference between 15, 30, 60 and 90 days post vaccination. For evaluation of protective immune response against rabies virus, indirect ELISA was found to be a reliable technique as per Piza *et al.* (1999) and sera with OD values greater than 0.282 were considered as corresponding to virus neutralising antibody > 0.5 IU/ml.

In a study on the serological response in cattle to tissue culture rabies vaccine, Ramanna and Srinivasan (1992) reported that the immune response of animals to vaccination with rabies vaccine varied widely, a few animals showed satisfactory immune response while small proportion of animals failed to elicit satisfactory antibody titres. This is an important factor while considering mass vaccination campaign wherein primary vaccination may consist of two vaccinations at a short interval. This will ensure most of the animals developing antibody titre to a satisfactory level. Sihvonen *et al.* (1994) worked on the immunisation of cattle against rabies using inactivated cell culture vaccine and reported that the rabies neutralising antibody titre (³ 0.5 IU/ml) was detected in 80 per cent of the animals tested after primary vaccination. Their results indicated that booster was always necessary after primary vaccination to ensure that all animals were protected.

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